A. Legal Standard

To anticipate a claim, the reference must teach every element of the claim. (M.P.E.P. § 2131.) "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v Union Oil Co. of California*, 814 F.2d 628,631 (Fed. Cir. 1987). "The identical invention must be shown in as complete detail as is contained in the. . . claim." *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236 (Fed. Cir. 1989). Further, a prior art reference must be considered in its entirety, i.e. as a whole, including portions that would lead away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540 (Fed. Cir. 1983).

B. The Claimed Invention

The claimed invention is directed to DNA/polyethyleminine(PEI)/hydrophilic polymer complexes wherein the hydrophilic polymer is covalently attached to the PEI.[1] The PEI of the invention is described in the specification at pages 5-6. Examples of PEI within the scope of the invention are disclosed at page 6 of the specification. From these examples, it is clear that the PEI of the claimed complex is a normal branched chain or linear polymer, which is typically available commercially. (*See e.g.* Sigma Product Information (Exhibit A)). Moreover, the use of the term "PEI" in the art, without further description, refers to normal branched chain or linear polymers rather than "starburst" polymers or hyper comb-branched polymers. Neither Yin *et al.* nor Tomalia *et al.* teach the use of normal branched chain polymer or linear PEI.

C. Yin et al.

Yin *et al.* teach hyper comb-branched polymers. Hyper comb-branched polymers comprise successive generations of branches branching off of prior generations of branches resulting in a

[1] In the interest of simplicity, these complexes will hereinafter be referred to as DNA/PEI/polymer complexes or DNA/PEI/hydrophilic polymer complexes.

structure with an exceedingly high degree of branching. (Column 3, lines 33-49.) The structure of the Yin *et al.* polymer is characterized by an exterior surface formed by the termini of the last generation of branches, and interior voids within the polymer molecule. (*Id.*) Applicants' claimed invention does not employ the hyper comb-branched polymers of Yin *et al.* Applicants' claimed invention comprises normal branched chain or linear PEI. Accordingly, Yin *et al.* do not teach each element of the claims.

The Examiner contends that Yin *et al.* teach "complexes comprising a nucleic acid and PEI, wherein PEI is covalently modified with a hydrophilic polymer." (Paper No. 6, page 5.) Applicants note, however, that Yin *et al.* teach a large genus of generic complexes that can *potentially* be formed, of which the complex referred to by the Examiner is a small subset. The Examiner has postured no evidence as to why one skilled in the art would be lead to the particular complex referred to by the Examiner among the many disclosed in the application.[2] In fact, Yin *et al.* teach away from use of the type of complexes employed in Applicants' claimed invention. Yin *et al.* observe that the type of PEI used in the Applicants' claimed invention is "not likely [to] be feasible. . .for gene transfection" due to the "relatively high toxicity of the polymers." (Column 32, lines 1-4.)

Yin *et al.* also teach that the hyper comb-branched polymers can be modified with a number of hydrophilic or hydrophobic polymers, including polyethylene glycol. (Column 5, lines 46-51.) However, Yin *et al.* do not specifically teach covalent attachment of polyethylene glycol to PEI polymers. Yin *et al.* do disclose examples of hyper comb-branched polymers that have PEOX grafted thereto. (Yin *et al.* column 30, line 66 to column 31, line 4.) These performed poorly in transfection studies when complexed with DNA leading Yin *et al.* to conclude that *amino*-containing groups should be grafted onto the hyper comb-branched polymers in order to increase transfection efficiency. (Column 31, line 11-13.) Thus, Yin *et al.* suggest away from use of normal branched or linear PEI

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[2] This assertion also holds true for the Tomalia *et al.* reference.

and away from the use of PEG grafting thereto. For the above reasons, Yin *et al.* do not anticipate the claims.

D. Tomalia et al.

Tomalia *et al.* teach "dense star" polycation polymers. A dense star or "starburst" polymer of PEI is a dendritic polymer exhibiting regular dendritic branching formed by the sequential or generational addition of branched layers to or from a core. (Column 2, lines 32-45.) Dendritic polymers encompass dendrimers, which are characterized by a core, at least one interior branched layer, and surface branched layers. (*Id.*) These polymer constructs comprise a core that is a chemical structure *in addition* to PEI. Tomalia *et al.* do not teach normal branched chain or linear polymer PEI as Applicants' claimed invention. Further, Tomalia *et al.* teach that polycation polymers may be "coated" or "shielded" with PEG. (Column 22, lines 36-37.) Tomalia *et al.* do not teach or suggest *covalent* attachment of PEG to the polycation polymers of the invention, including PEI. Thus, Tomalia *et al.* do not teach every element of the claim.

Because neither Yin *et al.* nor Tomalia *et al.* teach every element of the claims, these references do not anticipate Applicants' claimed invention. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

E. Bogdanov et al.

The Examiner has rejected claims 35, 41-45, 49, and 52 under 35 U.S.C. § 102(e) as allegedly being anticipated by Bogdanov *et al.* (U.S. Pat. No. 5,871,710). Applicants respectfully disagree.

Bogdanov *et al.* teach delivery of platinum(II) compounds using graft copolymer adducts. Bogdanov *et al.* do not teach complexing of the graft copolymer adducts with DNA. Accordingly, Bogdanov *et al.* do not teach every element of the claimed invention and therefore it cannot anticipate the claims.

II. Claim Rejections under 35 U.S.C. § 103

The Examiner has rejected claims 44-51 and 58-64 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Yin *et al.* (U.S. Pat. No. 5,919,442) or Tomalia *et al.* (U.S. Pat. No. 5,714,166), in view of Szoka (U.S. Pat. No. 5,661,025). Applicants respectfully disagree.

A. Legal Standard

A finding of obviousness in view of prior art references requires that: (1) the prior art must have suggested to those of ordinary skill in the art that they should make the claimed composition or device or use the claimed method, as the case may be; and (2) the prior art must have revealed that in so doing, those of ordinary skill would have had a reasonable expectation of success. *In re Vaeck*, 947 F.2d 488, 493, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991); *In re O'Farrell*, 853 F.2d 894, 7 U.S.P.Q.2d 1673 (Fed. Cir. 1988); *In re Dow Chemical Co.*, 837 F.2d 469, 473, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988). The prior art must provide one of ordinary skill in the art with the motivation to make the modifications required to arrive at the claimed composition. *In re Lalu*, 747 F.2d 703, 705, 223 U.S.P.Q. 1257, 1258 (Fed. Cir. 1984). Further, a prior art reference must be considered in its entirety, i.e. as whole, including portions that would lead away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540 (Fed. Cir. 1983).

B. Rejection of Claims 44-51

Claims 44-51 are directed to DNA/PEI/hydrophilic polymer complexes wherein the polymer is covalently attached to the PEI and wherein the polymer is about 500D to about 20,000D. The molar ratio of polymer: primary amino groups/PEI is about 1:10 to about 10:1. The claims are also directed to DNA/PEI/polymer complexes wherein the PEI is modified with a cellular ligand including transferrin and EGF.

Yin *et al.* teach complexes comprising a nucleic acid and hyper comb-branched polymers, including among an assortment of other polymers, hyper comb-branched PEI. Although Yin *et al.* teach that the hyper comb-branched polymers can be modified with a polymer such as polyethylene glycol, Yin *et al.* do not specifically teach covalent attachment of polyethylene glycol to PEI. Moreover, Yin *et al.* do not teach normal branched chain or linear PEI polymers as Applicants' claimed invention. Szoka *et al.* do not teach the normal branched or linear PEI and do not teach covalent attachment of PEG to PEI. Thus, Szoka *et al.* do not cure the defects of Yin *et al.* Because the references in combination do not teach every element of the claims, a § 103 rejection is improper. In order to sustain a rejection under 35 U.S.C. § 103(a), the prior art references must teach or suggest all the claim limitations. *In re Royka*, 490 F.2d 981 (CCPA 1974); M.P.E.P. §§ 2142, 2143.03.

Further, there is no motivation to combine the two references. The Examiner alleges that there is motivation to combine Szoka *et al.* and Yin *et al.* because "one of ordinary skill in the art would be aware of the teachings of Szoka, and be motivated to use these molecular weights and ratios as a starting point in optimization of the complexes because the compositions of Szoka are very similar in structure and function to those of Yin." (Paper No. 6, page 7.) Applicants respectfully disagree.

Although the structures are arguably similar in structure, there are notable differences between the polymers. It was known in the art that the various polycation polymers, though similar in structure, have distinct chemical and physical properties. For example, the degree of cationic charge is important in the ability of polycation polymers to complex with DNA. Nitrogen atoms must be protonated to achieve cationic charge, so the cationic charge density is pH dependent. Due to their distinct structures, PEI and the polymers (polyamidoamine) of Szoka *et al.* are likely to have different levels of protonation at physiological pH.

The Examiner has also alleged motivation to combine the references by pointing to the alleged similarity of function of the polymers of Yin *et al.* and Szoka *et al.* Specifically, the Examiner states that the "intended use of the compositions is the delivery of nucleic acids to cells." (Paper No. 6. Page

7.) But as noted above, Yin *et al.* teach *against* use of the type of PEI employed in the claimed invention. The reference states that "randomly branched PEI polymers are "not likely [to] be feasible . . . for gene transfection" due to the "relatively high toxicity of the polymers." (Yin *et al.*, Column 31, line 60; and column 32, lines 1-4.) Thus, Yin *et al.* teaches away from the claimed invention. Further, as noted above, Yin *et al.* demonstrate that modification of the hyper comb-branched polymer with PEOX resulted in reduced transfection efficiency. (Column 30, line 66 to column 31, line 4.) They suggest modification with primary amines such as polyamidoamine. (Column 31, lines 11-14.) Thus, they teach away from modification with PEG. It is improper to combine references where the references teach away from their combination. *In re Grasselli*, 713 F.2d 731, 743 (Fed. Cir. 1983). For the reasons set forth above, Yin *et al.* in view of Szoka *et al.* do not render unpatentable Applicants' claimed invention.

Tomalia *et al.* teach complexes comprising a nucleic acid and "dense star" polymers. Tomalia *et al.* teach that the complexes may be "coated or shielded" with polyethylene glycol. (Column 22, lines 36-37.) Tomalia *et al.* do not teach *covalent binding* of polyethylene glycol to PEI and Tomalia *et al.* do not teach the use of normal branched chain or linear PEI polymers. Szoka *et al.* do not teach the normal branched or linear PEI polymers and do not teach covalent attachment of PEG to PEI. Thus, Szoka *et al.* do not cure the defects of Tomalia *et al.* Further, as noted above, there is no motivation to combine the two references due to the difference in properties of the polycations disclosed in each reference. Because the references in combination do not teach every element of the claims and because there is no motivation to combine the references, a 35 U.S.C. § 103 rejection is improper.

C. Rejection of Claims 58-64

Claims 58-64 are directed to a process for preparing DNA/PEI/hydrophilic polymer complexes characterised in that first DNA and PEI, optionally modified with a cellular ligand, are complexed by

mixing dilute solutions and then the hydrophilic polymer is bound to PEI. The claims are also directed to this process wherein the DNA concentration is about 5 to 50 μg DNA/ml. The solution in which complexing occurs can be deionised water. After complexing, the complexes of dilute solution can be adjusted to a concentration of about 200 μg/ml to 1 mg/ml, based on DNA.

The Examiner has stated:

> "It would have been obvious to one of ordinary skill in the art at the time of the invention to prepare the PEI/DNA/PEG complexes of Yin and Tomalia by first mixing the DNA and PEI, and then adding PEG. One would have been motivated to take this approach because Szoka teaches that PEG is useful as a masking agent which shields DNA from degradation." (Paper 6, pages 7-8.)

The defects of Yin *et al.* and Tomalia *et al.* and the failure of Szoka to cure these defects have been discussed above. Thus, the combination of these references to form a 35 U.S.C. § 103 rejection is improper. Moreover, neither Yin *et al.*, Tomalia *et al.*, nor Szoka *et al.* teach addition of PEG *after* complexing of DNA and PEI. Yin *et al.* teach attachment of PEG groups as a step in the synthesis process of the dendrimer polymers. They state that "particular functional characteristics may be incorporated in the polymer *during its assembly*." (Column 5, lines 36-38.) (Emphasis added.) Thus, Yin *et al.* teach attachment of PEG *before* complexing.

Tomalia *et al.* and Szoka *et al.* are completely silent as to when or how PEG should be added. Szoka *et al.* do teach that PEG may be useful as a masking agent which shields DNA from degradation but this does not suggest to one of ordinary skill in the art to modify with PEG after complexing of DNA and PEI as the Examiner contends. The Examiner has not shown any evidence that PEG attachment after complexing would have been routine or obvious if the goal was to mask the DNA. In fact, DNA masking may be more effective if PEG attachment onto PEI were to occur prior to complexing. In the alternative, modification with PEG before complexing may be equally effective as modification with PEG after complexing. There is simply no teaching either way in the references relied upon by the Examiner nor was it common knowledge in the art to perform the steps in the order utilized in the claimed invention. Thus, there is no support for the Examiner's assertion

that "motivation" to PEGylate after complexing is found because Szoka *et al.* teach that PEG masks DNA.

The Examiner also states that "[i]t would have been similarly obvious to use DNA concentrations of about 5-50 or 10-40 μg/ml at a salt concentration below physiological value in this process because Yin and Tomalia both teach the use of DNA at a concentration of 50 μg/ml in water for the formation of complexes. . . [and] [t]he use of deionised water is standard operating procedure in molecular biology. . . ." (Paper No. 6, page 8.) Applicants respectfully disagree.

Yin *et al.* and Tomalia *et al.* teach a DNA concentration of 50 μg/ml as the lower range (i.e. 50 μg/ml-500 μg/ml) of possible DNA concentrations. (Yin *et al.*, column 29, lines 39-41; Tomalia *et al.*, column 49, lines 4-5.) Both are silent as to use of concentrations below 50 μg/ml and there is no motivation in either of these references to use concentrations below those recited in the specifications of these references. Thus, it would not have been obvious to use the concentrations recited in the claims, which fall below those discussed in Yin *et al.* and Tomalia *et al.*

Yin *et al.* and Tomalia *et al.* also suggest use of water during complexing. (*See id.*) But they are silent as to use of deionised water or water that has a salt concentration below physiological value. As noted, the Examiner is of the position that it would have been obvious to use deionised water because it is "standard operating procedure" in molecular biology.

Depending on the particular application, deionised water is typically used in molecular biology as a *starting* solution for further chemical treatment. For example, in setting up a PCR reaction, one of ordinary skill in the art would start with deionised water, *but* then add buffer, primer, DNA polymerase, etc. Northern blot solutions and buffer for electrophoresis also exemplify applications in molecular biology where deionised water is subsequently treated with salts to make buffered solutions. In fact, buffer, which by definition is a solution of salts, is as commonly or more commonly used in molecular biology than plain deionised water, particularly when a chemical reaction is involved. Thus, contrary to the Examiner's contention, standard operating procedure in

molecular biology does not suggest use of unbuffered solutions of water. Furthermore, the specific examples of DNA complexing provided in Yin *et al.* and Tomalia *et al.* use buffered solutions of water. For example, Yin *et al.* employ buffered solutions including 20mM HEPES, 100mM KCl, 0.2 mM EDTA, 0.5 mM DTT, and 20% (v/v) glycerol. (Column 32, lines 31-33.) Tomalia *et al.* also teach the use of buffered solutions for complexing DNA and polymer including for example, 100mM NaCl and 10mM TRIS. (Column 112, Example 51.) When the references are viewed in their entirety, they teach away from use of unbuffered deionised water. Accordingly, it would not have been obvious to use unbuffered or plain deionised water as Applicants have taught. Moreover, Applicants have disclosed that complexing in deionised water leads to significantly better results than complexing in buffered solution. (Example 1 and Figure 1.) Thus, it is Applicants' disclosure that identifies a problem and sets forth a solution to that problem.

D. Rejection of Claims 63-64

The Examiner states that "further optimization of the complexes for the purposes of transfection is well within the ability of one of ordinary skill in the art, and could reasonably be expected to lead to compositions with the characteristics of claims 63 and 64, particularly in view of the suggestion by Tomalia to dilute the complexes to 1-10 μg DNA/ml." (Paper No. 6, page 8.) Applicants respectfully disagree.

Neither Yin *et al.*, Tomalia *et al.*, nor Szoka *et al.* teach the concentrations discussed in claims 63 and 64. Further, Tomalia *et al.* teach *dilution* of the complexes. Specifically, Tomalia *et al.* teach a "fifty-fold" dilution of the complexes suitable for pharmaceutical compositions and packaging. (Column 49, lines 37-43.) Applicants' claims are directed to further *concentrated* (twenty to 40-fold) solutions to of the DNA/PEI/hydrophilic polymer complexes. Tomalia *et al.* do not teach or suggest concentrating the complexes in solution. In fact, increasing the DNA concentration of the Tomalia *et al.* complexes would most likely lead to undesirable aggregation of the complexes. Further

concentration of the complexes is possible only through use of the teachings in Applicants' disclosure, i.e. PEG attachment after complexing in deionised water. Thus, it would not have been obvious to concentrate the DNA complexes to the concentrations taught in the specification.

E. Rejection of Claim 52

The Examiner has rejected claim 52 as being unpatentable over Yin *et al.* or Tomalia *et al.* either one in view of Szoka *et al.* and further in view of Bogdanov *et al.* (U.S. Pat. No. 5,871,710). Again, Applicants respectfully disagree.

Claim 52 is directed to DNA/PEI/hydrophilic polymer complexes characterised in that the PEI is bound to a cellular targeting ligand via the hydrophilic polymer.

The defects of Yin *et al.* and Tomalia *et al.* and the failure of Szoka *et al.* to cure the defects have been discussed above. Thus, the combination of these references to form a 35 U.S.C. § 103 rejection is improper. In addition, neither of these references teach a targeting ligand bound to a hydrophilic polymer which is bound to PEI. The further combination of Bogdanov *et al.* is improper because there is no motivation to combine this reference with the others. Bogdanov *et al.* teach graft copolymer adducts of poly-l-lysine and PEI for the delivery and sustained release of platinum (II) compounds. Due to the different structure and function of the complexes of Bogdanov *et al.*, one of ordinary skill in the art would not be motivated to combine the references. Thus, a 35 U.S.C. § 103(a) rejection is improper.

In view of the above, Applicants respectfully request that the Examiner reconsider and withdraw the rejections based on 35 U.S.C. § 103.

III. Rejection of Claims under 35 U.S.C. § 112, First Paragraph

The Examiner has rejected claims 53-57 and 65-68 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to

enable one skilled in the art to which it pertains to make and/or use the invention. The Examiner has stated that:

> "[d]ue to the lack of guidance and working examples in the specification, a skilled artisan would have to perform extensive experimentation in order to develop successful gene therapy protocols utilizing the claimed compositions."

(Paper No. 6, page 2.) Applicants respectfully disagree.

Initially, Applicants note that claims 53-57 are not directed to "pharmaceutical compositions" or "gene therapy protocols" as alleged by the Examiner. (Paper No. 6, page 1.) Rather, the claims are directed to "complexes" which contain therapeutically active nucleic acid. These complexes are not necessarily utilized in the context of gene therapy or "therapeutic use." The limitation "therapeutically active" serves only to further limit the nature of the nucleic acid which is employed in the complex. Applicants assert that the fact that a therapeutically active protein is expressed does not convert the claimed complex into a gene therapy method. For example, the complexes may be utilized to transfect therapeutic genes in *ex vivo* expression systems for high-level protein production. Thus, Applicants assert that the Examiner has mischaracterized the nature of the invention as it relates to these particular claims.

Claims 53-57 need only be enabled as DNA/PEI/polymer complexes. There is clearly sufficient support in the specification to enable a skilled artisan to make and use this invention. Further, PEI/DNA complexes were well-known in the art (*see, e.g.*, Boussif, O. *et al. Gene Therapy* 3(12): 1074-1080 (1996) and, generally, easy to use (Boletta, A. *et al.*, *Hum. Gene Therapy* 8:1243, 1249 (1997) (Exhibit B); *see also* Examples 1 and 2 in the specification). Working examples of the inventive complexes and protocols for producing them are provided in the specification. (*See e.g.*, Specification, Examples 1 and 2.)

Similarly, claims 65-68 recite the complexes of claim 53 in a pharmaceutical composition. These claims are not directed to a method of human gene therapy. For example, pharmaceutical compositions of the invention have been used in mice. As noted, PEI/DNA complexes were well-

known in the art and generally easy to use. Working examples of compositions comprising the inventive complexes are provided in the specification. (Examples 10 and 12.)

Assuming, *arguendo*, that claims 53-57 and 65-68 must be enabled for "therapeutic use", Applicants assert that claims 53-57 and 65-68 are enabled as such for the reasons provided below.

The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). Some experimentation is permitted so long as the experimentation necessary to practice the invention is not undue. *In re Angstadt,* 537 F.2d 498, 504 (CCPA 1976). Further, as long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. § 112 is satisfied. *In re Fisher*, 427 F.2d 833, 839 (CCPA 1970); M.P.E.P. § 2164.01(b).

Under the "Guidance and working examples in the specification" portion of the June 29, 2000 Office Action, the Examiner has stated that:

> "no evidence is presented which would convey to a skilled artisan that the art-recognized problems associated with gene delivery or gene expression have been solved, and the issue of expression after delivery does not appear to have been addressed."

(Paper No. 6, page 2.) Applicants respectfully disagree.

Applicants have solved a problem—forming a composition that can have high concentrations of DNA/PEI complex that do not aggregate. The Examiner has not provided any evidence that there are "art-recognized" problems associated with gene delivery using complexes of DNA and PEI even if gene delivery was what was being claimed.

The specification provides sufficient guidance to one skilled in the art how to make and practice the claimed invention. Claims 65-68 are directed to pharmaceutical compositions containing normal branched chain or linear PEI/therapeutically active DNA complexes, characterized in that the PEI is modified with a hydrophilic polymer covalently coupled thereto. Examples 1 and 2 clearly

demonstrate how to make the DNA/PEI/polymer complexes of the invention. Example 6 provides a protocol for transfection of the complexes (which were further modified with transferrin) into human cell line K562 and murine neuroblastoma cell line. Figures 7 and 8 shows successful transfection and expression of the reporter gene luciferase in each of these cell lines. Example 15 provides a protocol for transfection of the complexes (which were further modified with EGF) and expression of the luciferase gene in human cell line K562. Thus, numerous examples of successful transfection of the DNA/PEI/polymer complexes and expression of the gene contained therein are provided. (*See, e.g.* Specification, Figure 16.)

In addition, Example 12 of the specification provides a protocol for targeted gene expression in tumor tissue of mice using the complexes of the invention. The results demonstrate that "substantial reporter gene expression was found in the tumor and the tail" with "[o]nly minimal expression [] detected in the lungs" and "no expression at all [] found in the other organs." (Specification, page 33; *see also* Figure 13.) Tumor tissue specific expression of transfected genes constitutes "therapeutic use" particularly when the gene expressed is a "therapeutic" gene. Thus, examples of successful transfection of the DNA/PEI/polymer complexes and expression of the gene encoded by said DNA in therapeutically relevant regions of model animals are provided. This demonstrates a utility for the claimed complex—i.e. gene expression in mice and evidence of such reasonably correlates with successful treatment in higher animals (if the claims were being analyzed as a method of treatment).

Further, the specification teaches what factors and conditions may be important for successful use of the claimed invention including, for example, the N/P value (nitrogen to phosphate ratio) of the complex, concentration of DNA, molecular weight of the hydrophilic polymer, and degree of PEGylation (molar ratio of polymer: primary amino groups/PEI). In view of such detailed guidance, Applicants assert that any experimentation, if any, necessary to practice the invention would not be undue.

In discussing the state of the prior art, the Examiner has cited Verma, I.M. *et al.*, *Nature*, 389:239-42 (1997) and Anderson, W.F., *Nature* 392(Suppl.):25-30 (1998) to support the proposition that "successful implementation of gene therapy protocols was not routinely obtainable by those skilled in the art." (Paper No. 6, page 3.) As noted by the Examiner, Verma, I.M. *et al.* state that "[a]lthough more than 200 clinical trials are currently underway worldwide, with hundreds of patients enrolled, there is no single outcome that we can point to as a success story." Anderson, W.F. states that after reviewing over 300 clinical trials "there is still no conclusive evidence that a gene therapy protocol has been successful in the treatment of human disease." Neither of these references discuss the success of the general group of polycation polymer vectors of which the claimed invention is a member. Further, these references, at most, support the proposition that gene therapy protocols, as a group, have not reached success at the *clinical* and *commercial* levels. But lack of success at the clinical and commercial levels does not indicate that the state of the prior art was somehow lacking or in some manner, non-enabling for what is particularly being claimed.

In fact, the state of the prior art demonstrates successful application of gene transfection into mammalian cells using PEI/DNA complexes. PEI has been found to mediate gene transfer into a variety of cell types leading some researchers to call PEI/DNA complexes a "versatile vector for gene and oligonucleotide transfer into cells in culture and *in vivo*." *See* Boussif, O. *et al.*, *Proc. Nat. Acad. Sci. USA* 92:7297-7301 (1995). Abdallah *et al.*, *Hum. Gen. Therapy* 7:1947-54 (1996) (Exhibit C) have reported that PEI/DNA complexes were successfully utilized for "unprecedentedly high levels" of gene transfer into the mouse brain. Boletta *et al.*, *Hum. Gene Therapy* 8:1243-1251 (1997) have shown successful gene transfer into rat kidneys using PEI/DNA complexes. In fact, they conclude that the PEI/DNA delivery technique "is easy to perform" making it "particularly appealing for potential future applications in humans." (*Id.* at 1250.) Kircheis *et al.*, *Gene Therapy* 4:409-18 (1997) (Exhibit D) have demonstrated up to a 1000-fold increase in transfection efficiency when cell-binding ligands (transferrin and antiCD3 antibody) are incorporated into the complex. Covalent

PEGylation of the complexes, as taught in the specification, would only further increase the level of expression of the transfected gene as shown in the Applicants' application. The result would be increased therapeutic value.

Additionally, successful gene therapy has been documented by Losordo *et al.*, *Circulation* 98:2800-2804 (1998) (Exhibit E) (concluding that naked plasmid DNA is safe may lead to reduced symptoms and improved myocardial perfusion in selected patients with chronic myocardial ischemia); Tsurumi *et al.*, *Suppl. Circulation* 96(9): II-383-388 (1997) (Exhibit F) (demonstrating that IM injection of plasmid DNA encoding VEGF yields successful gene expression in acutely ischemic skeletal muscles and ameliorates hemodynamic deficits in the ischemic limb by augmenting angiogenesis); Isner *et al. Lancet* 348(9024):370-74 (1996) (Exhibit G) (suggesting that intra-arterial gene transfer of plasmid which encodes for VEGF can improve blood supply to the ischaemic limb); and Yu *et al.*, *Oncogene* 11:1383-1388 (1995) (Exhibit H) (finding that liposome-mediated E1A gene transfer significantly inhibited growth and dissemination of ovarian cells that overexpress HER-2/*neu*).

Given the guidance provided in the specification and the state of the prior art, one skilled in the art would be able to routinely make and use the claimed invention for "therapeutic use" (if the claimed invention need be enabled as such). Moreover, Applicants have provided numerous *ex vivo* and *in vivo* working examples of the invention. Accordingly, Applicants assert that it would not require undue experimentation to practice the invention. In view of the above, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

IV. Rejection of Claims Under 35 U.S.C. § 112, Second Paragraph

The Examiner has rejected claims 46-48 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Specifically, the Examiner has stated:

> "These claims recite complexes with a range of specific molar ratios of polymer to PEI, whereas the specification recites processes for making complexes in which the starting materials are present in amounts within these ranges. It is not clear that the reaction mixes disclosed in the specification give rise to complexes recited in the claims."

(Paper No. 6, page 3.) Applicants respectfully disagree.

The specification teaches that a weight ratio of PEG-5000 D derivative to PEI of 9.2 was chosen as the starting point for determining the ratio of PEG to primary amino groups/PEI. (Specification, page 6, lines 31-33.) A weight ratio of 9.2 corresponds to a PEG: primary amino groups/PEI ratio of 1:1. The claims are limited to PEG: primary amino groups/PEI ratios from about 1:10 to about 10:1. Examples 1 and 2 of the specification teach use of 69 μg of PEG and 7.5 μg of PEI in the complexing solution. The PEG to PEI weight ratio of the complex in the example is 9.2. Thus, in Example 1, the PEG: primary amino groups/PEI ratio is 1:1, within the range recited in claims 46-48. In Example 6, complexes with PEG/PEI weight ratios of 2.3:1, 3.7:1 and 7.4:1 were created. Using the starting point described at page 6 of the specification, these weight ratios correspond to PEG: primary amino groups/PEI ratios of 0.25:1, 0.4:1 and 0.8:1. These ratios fall within the range recited in claims 46-48. Thus, the reaction mixes disclosed in the specification give rise to the complexes recited in the claims. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

The Examiner has rejected claims 58-63 under 35 U.S.C. § 112, second paragraph, because claim 58 recites "the dilute solutions" without antecedent basis. (Paper No. 6, page 3.) Applicants have amended claim 58 to provide antecedent basis for "dilute solutions." Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

The Examiner rejected claims 61 and 62 under 35 U.S.C. § 112, second paragraph, because claim 61 recites "the physiological value" without antecedent basis. (Paper No. 6, page 3.) The Examiner has also indicated that the claim does not make clear what salt concentration is being

evaluated. Specifically, the Examiner has questioned: "Is it NaCl, KCl, or some combination of salts?"

Applicants have amended claim 61so that it no longer recites "the physiological value." By the phrase "salt concentration," Applicants mean total salt concentration including KCl, NaCl, and any other salt that may be present in the solution. In view of the above, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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